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MILLENNIUM REVIEW

Gene therapy in the CNS

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Gene therapy for neurological disorder is currently an experimental concept. The goals for clinical utilization are the relief of symptoms, slowing of disease progression, and correction of genetic abnormalities. Experimental studies are realizing these goals in the development of gene therapies in animal models. Discoveries of the molecular basis of neurological disease and advances in gene transfer systems have allowed focal and global delivery of therapeutic genes for a wide variety of CNS disorders. Limitations are still apparent, such as stability and regulation of transgene expression, and safety of both vector and expressed transgene. In addition, the brain adds several challenges not seen in peripheral gene therapy paradigms, such as post-mitotic cells, heterogeneity of cell types and circuits, and limited access. Moreover, it is likely that several modes of gene delivery will be necessary for successful gene therapies of the CNS. Collaborative efforts between clinicians and basic researchers will likely yield effective gene therapy in the CNS. Gene Therapy (2000) **7**, 93–109.

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Goals of gene transfer to the CNS

Advancements in gene transfer technologies bring closer to reality the amelioration of disease states by introducing genes into the brain. Delivery of therapeutic genes can potentially protect against neurodegenerative disease or insults to the brain by delivery of growth factors, antioxidant, or anti-apoptotic molecules;[1,2] kill or slow the proliferation of neoplasms via transfer of therapeutic-enhancing proteins and anti-angiogenesis factors;[3–6] and down-regulate expression of dominantly acting gene products using antisense or ribozyme to the mutated mRNA, or compensate for loss-of-function mutations by protein replacement.[7] The genes and tools for delivery will need to be tailored to meet therapeutic goals. Treatment of a focal tumor or a cluster of cells within a discrete region of the brain may be more easily achieved than treating widespread abnormalities, and different diseases may require short-term or stable transgene expression. Several types of vectors based on modified viruses can mediate efficient short- and long-term expression within cells of the CNS after stereotactic delivery. Liposomes, genetically-engineered cells and direct DNA transfer have also shown potential in certain experimental paradigms. Unique attributes of the CNS, including the post-mitotic nature of neurons, heterogeneity of cell types, critical functions of specific neuronal circuits, limited access, volumetric constraints, and presence of the blood–brain barrier all present challenges not usually at issue in peripheral gene therapy.

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The implementation of gene therapy for acquired and inherited CNS disorders requires the continuing integration of different areas of expertise, including virology, neuroscience, neurosurgery, immunology, and molecular genetics. Many improvements in efficacy, stability, regulatability and safety of gene transfer to the brain are needed. First, a large transgene capacity is often desired to incorporate the gene(s) of interest and appropriate regulators or inducible promoters. Second, high transduction efficiency and titers are needed to express genes in specific populations of neural cells after stereotactic administration. Third, stability of transgene expression is required in many applications, and has been difficult to achieve due to promoter inactivation, physical loss of vector sequences, cytotoxic effects, and immune responses to foreign proteins, including transgene products and viral proteins.[7–9] Fourth, the appropriate levels of transgene product can be critical and inclusion of sequences within the vector to regulate transcription levels of transgene may be necessary for therapeutic control.[10–14] Fifth, the cell specificity of gene transfer within the nervous system (to neurons *versus* glia, and specific phenotypes of each) will depend on use of targeted vectors, which selectively infect particular cell types, cell-specific promoters,[15–18] and routing through neuronal projections in the brain.[19–21] Finally, for effective application of viral vector-mediated gene transfer for therapy, lack of toxicity and immune response will be essential, with the exception of brain tumors where these responses may be part of the therapeutic paradigm.[22] Although the brain is considered to have poor immunologic surveillance, inflammatory and immunological responses do occur, as documented with adenovirus (Ad) vectors,[8,23] and can cause damage to normal neural tissue.

The following sections provide an update on gene ther-

apy technology and delivery systems, and review recent work that applies these strategies to disorders of the CNS.

Tools for gene transfer to the CNS

HSV-1 recombinant virus and amplicon vectors

Herpes simplex virus type 1 (HSV) is a common pathogen in humans, causing primarily cold sores, but occasionally encephalitis and other life-threatening conditions, especially in immune-compromised individuals. It is an enveloped virus bearing 152 kb of double-stranded DNA encoding over 80 genes, which has high infectivity for neurons and glia, as well as many other cell types.[24] The virion enters the cell by fusion of the envelope with the plasma membrane, and the capsid is transported along microtubules to the nucleus. In neurons, HSV vectors are delivered by rapid retrograde transport along neurites to the cell body,[25,26] providing a means of targeting gene transfer to cells that are difficult to reach directly. The viral DNA is deposited in the nucleus, initially in a circularized episomal form, and eventually replicates, enters latency or is degraded depending on its composition. Two types of vectors are derived from HSV: recombinant virus vectors (RV) and amplicon vectors (for reviews see Jacobs *et al*[27] and Glorioso *et al*).[28] HSV-RV vectors contain the full viral genome mutated in one or more virus genes to reduce toxicity and provide space for transgenes (30–50 kb). Replication-conditional RV vectors can selectively replicate in and kill tumor cells in the brain (see Brain tumors below). Replication-defective RV vectors are designed to have minimal toxicity, and current versions delete multiple immediate–early genes that encode transactivating factors, thereby essentially eliminating expression of other viral genes, eg deletions of genes encoding ICP4, ICP22, ICP27 and ICP47 (the latter being involved in antigen presentation).[29,30] Elimination of ICP0 further reduces toxicity in some cells, but also results in low levels of transgene expression.[31] RV vectors can enter a stable, benign, episomal latent state in neurons, but with consequent down-regulation of most viral and cellular promoters. Long-term expression has been achieved in neurons using the LAT promoter(s) which are active in viral latency.[32–34]

The HSV amplicon vector consists of a plasmid bearing the HSV origin of DNA replication, *oris*, and packaging signal, *pac*, which allows it to be packaged as a concatenate in HSV virions in the presence of HSV helper functions.[35] These vectors can be packaged free of helper HSV virus by cotransfection with the HSV genome deleted for *pac* signals using a set of cosmids or BAC plasmid.[36–38] The advantages of these vectors are: essentially no toxicity or antigenicity, as they express no virus proteins,[39] albeit low-level contamination by recombinant replication-competent virus during packaging ($<1 \times 10^7$ transducing units (t.u.)/ml, and some virion proteins, eg VP16, that are toxic at high levels); large transgene capacity (demonstrated up to 22 kb,[40] and potentially up to 150 kb);[41] relatively high titers (up to 10^9 t.u./ml with current packaging modalities); high infectivity for cells of the nervous system; and retention for up to months in nondividing cells.

Adeno-associated virus (AAV) vectors

AAV consists of a non-pathogenic, small virion (20–24 nm in diameter) containing a single-stranded DNA genome. AAV-based vectors have a 4.5 kb transgene capacity[42] and inverted terminal repeats (ITRs) that promote extrachromosomal replication and genomic integration of the transgene.[43] In wild-type AAV infections, the *rep* gene encodes a set of Rep proteins which mediate the replicative amplification of the ITR-flanked genome and facilitate integration into the host cell genome. Integration of transgenes delivered by AAV vectors can be random or site-specific into human chromosome 19q13.3.[30,44–46] Long-term expression of transgene from AAV-based vectors is facilitated both by integration and maintenance as an episomal element within the host cell nucleus.

The replication and packaging of AAV vectors previously required the presence of adenovirus (Ad) helper virus to provide packaging functions, which was later heat inactivated or separated by density gradient centrifugation; however, denatured Ad proteins are a potential source of toxicity (reviewed in Ferrari *et al*).[47] A recent study has described an Ad-free method for producing recombinant AAV at high titers.[50] In addition, new strategies for purification of AAV vectors have been developed, based upon the identification of heparan sulfate proteoglycan as a cellular receptor for AAV[51] and specific antibodies that recognize AAV virions,[52] eliminating the need for potentially toxic reagents (such as CsCl) for generating purified vector.

The specificity and stability of expression from AAV vectors seems to be dependent upon the brain area and the presence of AAV receptors on target cells. AAV-based vectors produce high levels of transgene expression initially after injection into the CNS, predominantly in neurons.[53–55] As with other vectors, diffusion is limited and increases only modestly with larger injection volumes.[56] Time-course studies have shown reductions in numbers of transgene-positive cells over several months in some brain regions, with sustained expression in other regions.[16,54–57]

Strategies to more specifically target AAV transduction include the incorporation of nonviral ligand sequences into the AAV capsid, and the use of bispecific antibodies to target specific cellular receptors.[58,59] Pharmacological regulation of gene expression with AAV vectors has been obtained when regulatory elements and drug-responsive promoters are included.[60] For example, recent studies utilized a transgene under the control of a recombinant promoter that requires a reconstituted dimeric transcription factor complex to become activated.[14,61] Reconstitution of this complex depends upon the binding of a drug (eg rapamycin) to two chimeric proteins, thus allowing pharmacological regulation of the transgene expression.

Little toxicity has been observed with AAV vectors in brain and other tissues. Antibodies to AAV capsid proteins were low at 2 and 4 months after intracerebral injection and did not prevent transgene delivery upon readministration of AAV.[56] Pre-existing immunity to AAV caused by naturally acquired infections (antigen-specific immunity) has been studied in normal human subjects and cystic fibrosis patients; almost all had antibodies to AAV type 2, although most were not neutralizing, and only 5% of patients had peripheral lymphocytes that proliferated in response to AAV antigens.[7]

Adenovirus (Ad) vectors

The first generation of replication-defective Ad vectors constructed by deleting E1a, E1b and E3 genes, proved to have limited use in gene therapy, mainly due to a strong host immune response to the viral antigens.[62,63] New modifications of the vector have decreased the expression of viral proteins by deleting E2a and E4 genes, and/or specific open reading frames in the E4 gene.[64–66] Retention of E3 also decreases the antigenicity of the vectors.[67] *In vivo* experiments have shown long-term transgene expression and a lower level of inflammatory response in the host using E1/E4-deleted or E1/E2a-deleted vectors, as compared with E1/E3-deleted vectors,[68–70] though not all studies confirm this.[71]

Recently, high-capacity 'gutless' or 'mini-chromosome' Ad vectors have been generated that retain only the sequences necessary for packaging and replication of the viral genome, and lack all structural genes.[72–74] These gutless vectors have the advantages of increased transgene capacity (up to 37 kb) and propagation to high titers without contaminating helper Ad virus using a Cre–lox-based recombinase system.[74] *In vivo* studies have shown prolonged expression of transgenes delivered by these vectors with low host inflammatory response.[75–77] Even in the presence of peripheral infection with adenovirus, there is virtually no immune response in the brain following direct injection of gutless vectors in rats.[78] Another feature of both E1-deleted and gutless adenovirus vectors is their capacity to integrate randomly into the human and non-human chromosome.[79,80] However, integration events occur at low efficiencies, and integrated vector sequences have a propensity for rearrangements. The high antigenicity of the Ad virion and toxicity of the virion penton protein[81] remain as potential complicating factors with this vector system.

Retrovirus vectors and ex vivo delivery

Retrovirus vectors are derived primarily from Moloney murine leukemia virus (MoMLV).[82] These are enveloped RNA viruses which can transfer genes to a wide spectrum of dividing cell types.[83] Vector production utilizes packaging cells which express the retroviral *gag–pol–env* genes and can continually release vectors. The vectors bear up to 8.5 kb of transgenes flanked by retroviral long terminal repeat (LTR) regions, a virion packaging signal (psi), and a primer binding site for reverse transcription. Retroviral RNA within the cell is reverse transcribed into double-stranded DNA and these sequences integrate randomly into the host cell genome. The use of retrovirus vectors for gene delivery to the nervous system has been limited by their ability to transfer genes only to dividing cells, yet have been well suited for on-site delivery to neural precursors for lineage studies[84] and to tumor cells for therapeutic intervention (see Brain tumors below), and for *ex vivo* transplantation strategies.

The *ex vivo* approach to gene transfer has utilized a variety of cell types, including fibroblasts, astrocytes, endothelial cells, and neural progenitor cells. These cells can be maintained and genetically transduced in culture and then transplanted into diseased brain, serving as biologic 'minipumps' for gene products (reviewed in Bankiewicz *et al*[85] and Raymon *et al*).[2] Cells have been engineered to secrete trophic factors, neurotransmitters and metabolic enzymes by transfection via retroviral vectors or plasmids (reviewed in Martinez-Serrano[86]). These pump systems have proven effective in some animal models,[87] but issues remain: it has been difficult to obtain stable, regulatable secretion of gene products *in vivo*; immortalized cell lines have the potential to form tumors and disrupt host circuitry; and ethical issues confound with the use of human fetal tissue.

Hybrid vectors – HSV/AAV hybrid amplicon vectors

Since the current vector systems do not reproducibly achieve stable gene delivery to the CNS, and since readministration of vector into the brain would pose high risks to patients, hybrid (chimeric) viral vector systems are currently being developed that incorporate different viral elements to stabilize the transgenes. For example, critical elements from HSV amplicon and AAV vectors have been combined to produce HSV/AAV hybrid amplicon vectors.[88] These hybrid amplicons contain signals for propagation in bacteria, as well as the HSV-1 *oris* and *pac* elements. The transgene is flanked by the ITR sequences from AAV, and hybrid vectors have been produced both with and without the AAV *rep* gene to evaluate its importance in producing sustained transgene expression.[88] Hybrid amplicons have been constructed with over 20 kb of transgene sequences,[40,89] and grown to high titers (10^6 t.u./ml). These vectors can extend transgene expression in dividing human glioma cells well beyond the capacity of HSV amplicons.[88]

Transduction efficiency in primary neuronal cultures by HSV-derived amplicon vectors, packaged via a helper virus-free system,[36] was significantly higher than for AAV and Ad vectors at the same MOI.[39] Further, hybrid vectors mediated longer expression of the transgene in neurons, as compared with AAV and Ad vectors. Whether this is a result of replicative amplification or integration of the ITR-flanked transgene is currently under investigation in similar systems.[45,90] One month after injection of hybrid amplicon vectors into rat striatum, transduction efficiency was similar to (and in many cases higher than) basic HSV amplicon vectors. Transgene-expressing neurons were observed in both the striatum (over 8100 striatal cells, predominantly neurons) and the substantia nigra (over 500 nigrostriatal neurons, through retrograde transport ipsilateral to the injection site) per 10^6 t.u./ml injected (Figure 1). Of note, there was no immune response (analyzed via specific immune-response markers for T cells and microglia), or inflammation caused by these vectors.[39] The helper virus-free HSV/AAV hybrid amplicon vectors retain the retrograde transport and large transgene capacity of HSV, long-term transduction potential of AAV, and the ability to infect both dividing and nondividing cells, nonpathogenicity, low immunogenicity, and high titer capabilities of both vectors.

HSV/Epstein–Barr virus (EBV) and HSV/EBV/retrovirus hybrid amplicon vectors

The HSV/EBV hybrid vectors contain, in addition to HSV amplicon elements, two elements of Epstein–Barr virus (EBV): the latent origin of DNA replication, ori-P, and the nuclear antigen 1 gene, EBNA-1.[91] The EBV elements mediate episomal replication and chromosome retention over extended periods in dividing cells.[91,92] Starting with an HSV/EBV backbone, Sena-Esteves *et al*[89] incorporated *gag–pol–env* sequences and retrovirus vector sequences to form a 'tribrid' amplicon vector. This tribrid vector can

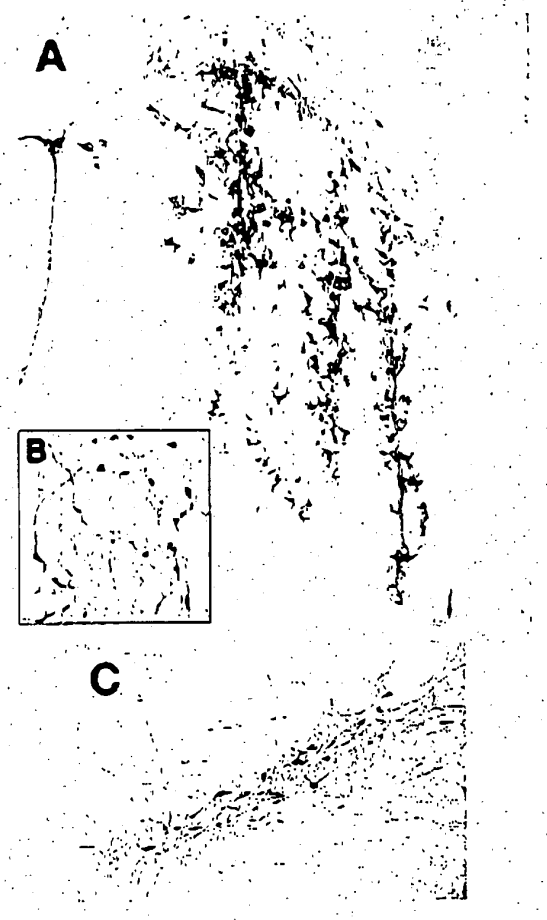

Figure 1 Gene transfer by HSV/AAV hybrid vectors of GFP to the nigrostriatal system. (A) Striatal cells expressing transgene (GFP immunostaining) 1 month after intrastriatal injection of vector (original magnification × 5). Three needle passes are visible in this representative plane of section. (B) Morphology of transduced striatal neurons (GFP immunostaining) approximately 100 μm away from needle track, 1 month after instrastriatal injection of HyRC (original magnification × 80). (C) Neurons within the SN expressing transgene (GFP immunostaining) 1 month after intrastrintal injection of vector (original magnification × 80). Note the large soma and morphology typical of nigrostrintal DA neurons. Reproduced from Costantini et al.[39]

convert both dividing and nondividing cells to retrovirus vector producer cells in a single infection step, allowing continuous production of retrovirus vectors at titers of 10^5 t.u./ml for 20–30 cell divisions in some cells. This mode of retrovirus vector delivery can potentially be utilized to facilitate on site gene delivery to dividing cells *in vivo*, such as tumor cells and neuroprogenitor cells via endogenous or transplanted cells converted to retrovirus-vector producing cells by amplicon infection.

Ad/AAV hybrid vectors

Exploiting the high efficiency and large cloning capacity of Ad vectors, and the integration capabilities of AAV, a set of Ad/AAV hybrid vectors have been produced: one encoding the AAV Rep78 protein and the other containing an ITR-flanked transgene.[93] Infection of human hepatoma cells yielded transgene integration at the AAVS1 site, but this system is limited by the need for co-incident infection by two Ad vectors. Another form of this Ad/AAV hybrid consists of Ad containing an AAV ITR-flanked transgene, in which the AAV Rep isoforms are introduced as extraviral components by conjugating them to the virion via a poly-L-lysine bridge.[94]

Ad/retrovirus hybrid vectors

A chimeric system that combines the high efficiency of Ad vector with the integrative capacities of retrovirus has also been produced.[95,96] The target cells are converted into transient retroviral producer cells via co-infection with two types of Ad vectors that deliver retroviral packaging functions and retroviral vector/transgene sequences, respectively. The progeny retroviral vectors are released from the producer cells *in situ*, infect neighboring cells, and lead to integration of the transgene. Successful expression of marker transgenes has been obtained in culture and in tumors *in vivo* with this system.

Lentivirus vectors

The main advantage of lentivirus-based vectors is their ability to integrate into the host genome of nondividing cells, thereby providing the potential for a delivery system with stable expression even in post-mitotic neurons.[97] The restricted host range, low titers, and pathogenic characteristics of HIV-1, itself, limit its utility as a gene delivery system for the CNS. In an effort to retain the positive attributes of HIV-1 and produce a safer and more versatile system, HIV-based vectors have been genetically manipulated to produce the lentiviral vectors currently being utilized. The HIV-1 vector is pseudotyped with the vesicular stomatitis virus G glycoprotein (VSV-G), broadening the host range to include brain, liver and muscle cells.[97–100] To minimize the possibility of generating replication-competent virus through recombination, a three-plasmid expression system is used, consisting of an HIV-1 packaging plasmid, a vector plasmid containing viral integrase and promoter-driven transgenes, and a plasmid expressing the surface VSV-G glycoprotein.[99] The generation of a tetracycline-inducible VSV-G pseudotyped lentivirus packaging cell line allows production of virus particles greater than 10^6 infectious units (IU)/ml for at least 3 to 4 days.[101] To enhance the safety of this system further, a self-inactivating (SIN) lentivirus vector has been constructed. The U3 region of the 5′ LTR was replaced with the CMV promoter, and the U3 region of the 3′ LTR (containing TATA box and transcription-factor binding sites) was deleted.[102] In addition, inclusion of polyadenylation sequence in the U5 region of the 3′ LTR increased vector titers.[103] Further manipulations to increase safety include encapsidation of the HIV-1-derived genome using a nonvirulent strain of simian immunodeficiency virus (SIV) to reduce the possibility of generating replication-competent HIV.[104]

The intracerebral delivery of genes via lentivirus vector has shown impressive results. After intrastriatal injection, the number of transduced cells (predominantly neurons) was significantly higher than with AAV and retrovirus vectors at all time-points, and higher than Ad at later time-points (24 weeks).[105] Transgene expression was also

observed in secondary sites due to retrograde transport, albeit to a lower degree than observed with HSV and Ad. Long-term expression from these vectors has also been shown, presumably due to insertion of the transgene into host genome, and no significant immune response.[105] Injection of SIN lentivirus vector into rat brain showed neuronal transduction as efficient as with original lentivirus vector at 2 and 6 weeks after infection, with indications of a broader range of expression in different cell types, possibly due to removal of the influence of the LTR on the internal CMV promoter.[102]

Liposomes and direct DNA transfer

Nonviral gene delivery systems include naked DNA, cationic lipids and polycationic polymers. Naked DNA consists of a plasmid DNA expression cassette that is directly delivered into the tissue by injection or particle bombardment[106] and enters the cytoplasm and subsequently the nucleus by means of endocytosis or transient membrane disruption.[107] Cationic polypeptides (polylysine, spermidine) bind to and condense negatively charged DNA, and are often linked to cell surface binding ligands. Coupling polylysine to a specific ligand, eg transferrin or insulin, induces endocytosis and targets DNA transfer to specific cell types.[108,109]

Cationic lipids condense and encapsulate DNA in positively charged complexes (liposomes) that enter the cell by endocytosis. To avoid degradation by the endocytic pathway and to facilitate fusion with the cell membrane, fusion proteins derived from Sendai virus have been incorporated into liposomes.[110,111] To facilitate cytoplasmic transit to the cell nucleus, high mobility group proteins with nuclear localization signals have been complexed to the DNA.[112,113] Most recently, elements from Epstein–Barr virus (oriP and EBNA-1) have been included in the DNA within liposomes to prolong the retention of transgenes in dividing cells.[114]

Few studies have used naked DNA and cationic liposomes to transfer genes into cells of the rodent brain.[115,116] In the brain, as in peripheral tissues, nonviral vectors induce nearly no immune response or toxic effects. However, there is a low efficiency of expression of introduced genes compared with viral vectors.[116]

Routes of delivery into brain

Gene delivery to the brain presents several unique challenges: limited and risky access through the skull (thus limiting repeat injections); sensitivity to volumetric changes (thus minimizing the size of inoculums and presenting increased risk from inflammatory responses); critical functional nuclei controlling life functions (which must be protected); and a highly specialized blood–brain barrier (designed to prevent viruses from entering the brain). The heterogeneity of the CNS neurocircuitry provides the opportunity to target focal areas of disease pathogenesis, as seen, for example in Parkinson's disease, but further complicates delivery to more widespread CNS diseases, such as brain tumors and lysosomal storage diseases.

A number of modes of delivery have been developed to tackle both focal and global delivery using various vector and cell vehicle designs (for review see Rainov *et al*[117] and Muldoon *et al*[118]). Most gene delivery to the brain in animals has involved direct stereotactic injection of replication-defective vectors into the brain. By this route the vector, or other form of DNA, is taken up by cells only in the immediate vicinity of the injection site, as diffusion is limited, with slower injection rates allowing somewhat wider dispersion. Spread of vectors to many other brain regions can be mediated by anterograde or retrograde transport of vectors within neurons projecting to the injection site (Figure 2). Newer modalities of delivery have included generation of vectors on site by injection of packaging cells to produce retrovirus vectors, which, in turn, infect residually dividing cells in the brain, including glia and neuroprogenitor cells, as well as tumor cells,[119] and by allowing limited replication of HSV or adenovirus vectors, used mostly in the context of tumors where toxicity is a component of the therapy.[120,121] On site vector generation can potentially be combined with the use of migratory cells, such as neuroprogenitor cells[122,123] or endothelial cells,[124] to increase the range of gene delivery in the brain.

Other routes of delivery to cells in the CNS have included: injection into fluid spaces, such as the vitreous humor in the eye;[125] or the CSF through intrathecal or intraventricular routes for delivery to the choroid plexus, ependymal/meningeal layers, and from there into the adjacent brain through processes extending into these layers;[126,127] and passage across the blood–brain or blood–tumor barriers by intra-arterial injection combined with temporary osmotic[128,129] or pharmacologic[130–132] disruption. Brain tumors can be preferentially targeted due to the relatively high permeability of the tumor neovasculature, as compared with the blood–brain barrier that has tight junctions between endothelial cells and is surrounded by an astrocytic sheath.[133]

Studies have begun to try to restrict the cell types expressing the transgene within the zone of delivery. This can be achieved in three basic ways: (1) by taking advantage of intrinsic cell properties, eg only some glia, neuro-

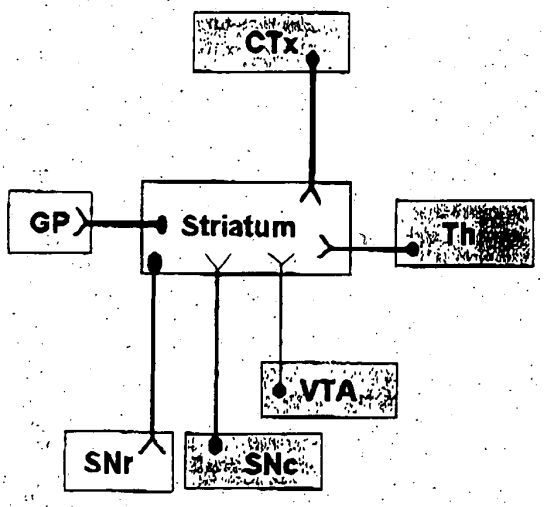

Figure 2 Schematic diagram of major afferent and efferent projections and from the striatum. Bold lines represent the strongest projections. CTx, cortex; GP, globus pallidus; SNc, substantia nigra pars compacta; Snr, substantia nigra pars reticulata; Th, thalamus; VTA, ventral tegmental area. The routes followed by vectors vary among virions and strains (eg Margolis et al).[243]

progenitor cells and tumor cells divide in the adult brain and thus can integrate and express transgenes delivered by retrovirus vectors; and only neurons have retrograde and anterograde viral transport mechanisms, so that at areas distant from the injection site only projecting neurons will be labeled; (2) by modifying the vector coat or virion such that it binds to or enters only specific cell types, eg Ad virions expressing ligands for or antibodies to the EGF receptor will preferentially infect tumor cells in the brain which overexpress these surface receptors;[134] and (3) by using cell specific promoters, eg the tyrosine hydroxylase promoter (to confine transgene expression to neurons which use dopamine or norepinephrine as transmitters)[123] the preproenkephalin promoter,[51] and the glial fibrillary acidic protein (GFAP) promoter for astrocytes and some gliomas.[126]

Applications for gene transfer to the CNS

Strategies

A number of experimental animal models have been utilized in gene therapy paradigms for disease states, including neurodegeneration, trauma, pain and ischemia, in which damage is usually induced by lesion or drug treatment, and brain tumors, in which cultured tumor cells are implanted in the brain. The applicability of gene therapy strategies developed in animals models for human disease will depend in large part on whether the etiology and pathogenesis is similar in these species. As more neurologic disease genes are identified, it is becoming possible to use existing mouse mutants and to generate transgenic mouse models which have a similar genetic etiology to human disease, and sometimes have the same phenotype. Such mouse models include lysosomal enzyme deficiency states, retinal degeneration syndromes, several forms of epilepsy, and, to an increasing extent, spontaneous tumor formation and neurodegenerative syndromes. Therapeutic strategies for neuronal rescue have three basic modalities: (1) decrease the action of a dominant-negative mutant protein by antisense or ribozymes; (2) replace missing enzyme or protein function in recessive conditions; and (3) provide general support to neuronal survival with protective proteins (trophic factors, anti-oxidative enzymes, chaperone/heat shock proteins) and anti-apoptosis factors. For brain tumor therapy, strategies include viral lysis, activation of cancer drugs, anti-angiogenesis, inhibition of tumor cell migration, immune enhancement and induction of apoptosis. The greatest limitations to successful therapy, even in experimental animals, are difficulties in achieving efficient gene delivery to sufficient numbers of target cells (which will be an even greater problem in human brain); loss of transgene expression over time; and direct or immune-related toxicity of vectors or transgenes.

A growing understanding of the role that defective genes play in the etiology of neurologic disorders emphasizes the need selectively to block the biosynthesis of harmful proteins in the brain. Diseases resulting from dominantly acting mutant proteins may respond to therapy by suppressing or blocking expression of these proteins via antisense oligonucleotides and ribozymes designed to block translation of specific RNA species.[137] A recent study infused antisense phosphorothioated oligodeoxynucleotide (s-ODN) into the cerebral ventricle of rats, and observed time-dependent diffusion and cellular uptake gradients in the hippocampus and cortex, and successful inhibition of specific gene products as well as selected downstream events.[138] Delivery of ODNs can be problematic, however, since they are unable to cross the blood–brain barrier. Methods to protect the nucleotides from degradation include biotinylation at the 3′ terminus of PO-phosphorothioate(PS)-ODN; and transport into the brain has been enhanced by a conjugate delivery vector consisting of streptavidin and a monoclonal antibody (OX26) directed to the transferrin receptor.[139] Additional delivery methods for antisense include expression constructs encoding antisense transcripts complexed with a cationic polymer, polyethylenimine.[140] Such constructs encoded in vectors could potentially allow stable, on-site inhibition of mutant protein expression.

Parkinson's disease (PD)

Two main strategies have been tested in gene therapy models for neurodegenerative diseases like PD: transfer of genes encoding neurotransmitter-synthesizing or metabolic enzymes to enhance the function of partially degenerated systems, and transfer of trophic factors and protective proteins to slow or halt the continuing neurodegenerative process (Table 1). The progressive loss of dopamine (DA) neurons within the substantia nigra and the resulting decrease of DA levels within its striatal target result in the motor symptoms characteristic of PD. Long-term use of the primary pharmacological agents used to treat PD, the DA precursor L-dopa, brings side-effects that eventually outweigh the benefits, prompting interest in developing new therapeutic strategies for this disorder. The focal region and cell specificity of degeneration in PD make this disorder an attractive candidate for therapeutic strategies that transfer genes for enzymes involved in the synthetic pathway of DA, and trophic factors to block the degeneration of the nigrostriatal system.

Since the loss of DA underlies the motor abnormalities in PD, several lines of investigation have focused on replacing DA levels. In addition to the well-known animal and human studies utilizing the transplantation of fetal DA neurons (reviewed in Dunnett and Bjorklund[141]), the transfer of genes involved in DA biosynthesis seeks to alter the phenotype of striatal neurons to allow them to produce DA. Transfer of tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of DA, to striatum has been extensively investigated. Procedures include direct transfer *in vivo* of the TH cDNA to striatal cells via viral vectors,[51,135,142–144] transplanting cells which have been transduced *ex vivo* to express TH,[87,145–150] and administration of a DNA–liposome complex encoding TH.[151–153] More recently the full pathway of DA synthesis been included in this approach, with the additional transfer of the synthetic enzyme GTP-cyclohydrolase (GTPCH) to generate the TH cofactor, tetrahydrobiopterin,[55,154–156] as well as transfer of aromatic amino acid decorboxylase (AADC) to facilitate conversion of L-Dopa to DA.[157–159] Together these genes may maximize production of DA in the striatum. Delivery of AADC may also allow regulation of DA levels in combination with titrated peripheral delivery of L-dopa.

Determination of functionality of the transferred genes has proven ambiguous, mainly due to limitations of the parkinsonian animal models. Apomorphine-induced rotation in the unilateral 6-OHDA lesion model is

Table 1 Gene therapy for PD

Viral			Other		
Gene	*Delivery*	*Author/Ref.*	*Gene*	*Delivery*	*Author/Ref.*
Replacement			*Replacement*		
TH	HSV	During *et al*[143]	TH	Fibroblasts	Fisher *et al*[17]
TH	AAV	During *et al*[143]	TH	Astrocytes	Fitoussi *et al*[145]
TH	Ad	Horellou *et al*[144]	TH	Cells	Horellou *et al*[146]
TH	HSV	Jin *et al*[155]	TH	Astrocytes	Lundberg *et al*[147]
TH	AAV	Kaplitt *et al*[54]	TH	C6 cells	Trejo *et al*[148]
TH/GTPCH	AAV	Mandel *et al*[55]	TH	Fibroblasts	Wolfe *et al*[149]
TH/AADC	AAV	Fan *et al*[157]	TH	Liposome	Cao *et al*[151]
AADC	AAV	Leff *et al*[156]	TH	Liposome	Imaoka *et al*[152]
			TH	Liposome	Segovia *et al*[153]
			TH/GTPCH	Fibroblasts and 9L gliosarcoma	Leff *et al*[156]
			TH/AADC/GTPCH	Fibroblasts	Wachtler *et al*[154]
			AADC/VMAT[a]	Fibroblasts	Lee *et al*[150]
Protection			*Protection*		
GDNF	Ad	Bilang-Bleuel *et al*[19]	BDNF	Fibroblasts	Galpern *et al*[169]
GDNF	Ad	Choi-Lundberg *et al*[166]	BDNF	Fibroblasts	Levivier *et al*[170]
GDNF	Ad	Lapchak *et al*[167]	GDNF	Encapsulated BHK cells	Lindner *et al*[171]
GDNF	AAV	Mandel *et al*[168]	BDNF	Astrocytes	Yoshimoto *et al*[172]
bcl-2	HSV	Yamada *et al*[174]	BDNF	Fibroblasts	Frim *et al*[173]

[a]VMAT: vesicular monoamine transporter involved in DA storage by packaging DA into synaptic vesicles and regulating release of DA.

reported to be decreased in several studies, suggesting recovery of striatal DA levels and tone.[142,144] However, damage to the striatum due to the transduction procedure itself or toxic/inflammatory reactions can also decrease apomorphine-induced rotations.[160] Other behavioral paradigms have been developed for the rat PD model,[161,162] and new models include a DA-deficient mouse that is dependent upon L-dopa for survival.[163] 'Rescue' of these mice (survival without administration of L-dopa) was achieved with delivery of TH and GTPCH to the striatum via AAV vectors.[164] Although clearly indicating functional delivery of genes involved in DA production, this is not an accurate model of PD, since the cellular nigrostriatal system remains intact in these mice.

Preservation of the nigrostriatal system by protection of DA neurons in the substantia nigra is the optimal goal in PD, both to slow the progression of the disease and to decrease the complications that arise with L-dopa therapy. In the protection paradigm, genes encoding trophic factors, anti-apoptotic molecules and anti-oxidants are transferred to the nigrostriatal system, utilizing both *in vivo* and *ex vivo* approaches (Table 1). The potent actions of glial-derived growth factor (GDNF) and brain-derived growth factor (BDNF) on the nigrostriatal system have prompted development of vector-mediated modes of delivery of these factors in PD brain models,[165] as these trophic peptides cannot pass through the blood–brain barrier after systemic administration, and complications such as gliosis and inflammation can arise from indwelling cannula. Promising results have been obtained with both factors, particularly after viral vector delivery to the striatum or substantia nigra,[19,166–168] but also after grafting of genetically modified cells.[169–173] Other factors with protective or regenerative effects include antiapoptotic genes, such as bcl2,[174] and enzymes that produce antioxidant molecules, such as superoxide dismutase. In the case of PD, long-term expression of the transferred gene may be required over the subsequent lifetime to protect against the progressive nature of degeneration.

Huntington's disease (HD)

Autosomal dominantly inherited HD is a potential candidate disorder for gene therapy, as a single type of mutation in the IT15 gene causes an expansion of the polyglutamine tract in the huntington protein. Although the normal function of IT15 has yet to be defined, evidence indicates a 'gain of function' of the mutated protein (for review see Wexler *et al*[175]), conferring increased susceptibility to excitotoxic damage, oxidative stress, and mitochondrial dysfunction in certain neuronal populations. Several molecular approaches for gene therapy of HD have been investigated at the DNA, RNA and protein levels (reviewed in Isacson[176]). Anti-sense strategies have proven technically difficult for this disorder,[6] but protective strategies have shown promising results.

Involuntary movements associated with the disease reflect predominantly GABAergic degeneration in the caudate and putamen, although cortical pathology is also present.[177] Protective gene therapy for HD have included transplanting cells engineered to secrete protective and replacement factors (reviewed in Kordower *et al*[178]), such as NGF-producing fibroblasts[179] and neural progenitor cells derived from transgenic mice in which the GFAP promoter directs the expression of human nerve growth factor (hNGF).[180] When transplanted into the quinolinic acid-lesion animal model of HD, these engineered progenitor cells reduced the size of lesion, spared striatal neurons, induced sprouting of cholinergic fibers from basal forebrain neurons, and reduced the astrocytosis

seen in lesioned animals. In a nonhuman primate model of HD, monkeys received intrastriatal implants of polymer-encapsulated fibroblasts, which had been genetically modified to secrete ciliary neurotrophic factor (CNTF), a trophic factor for striatal neurons, before intrastriatal lesion. These encapsulated cells protected striatal neurons that normally degenerate after lesion, and prevented retrograde atrophy in cortex and striatal target regions.[181] Protection via caspase inhibition has been observed when a transgenic mouse model of HD was crossbred with a transgenic mouse expressing a dominant-negative mutant of caspase-1.[182] These mice showed delayed onset of motor deficits, reduced formation of neuronal intranuclear inclusions, and increased longevity when compared with transgenic HD mice.

One issue that arises when considering gene therapy for HD is when the therapy should be administered, and how to achieve more global delivery. Since the defect can be detected early in life, intervention may be plausible. Functional imaging techniques of PET and SPET in HD carriers have shown reduced striatal glucose metabolism and DA receptor binding in all symptomatic and approximately 50% of asymptomatic adults, with a 30–40% loss of striatal DA receptor binding correlating with the emergence of symptoms, thus revealing a window for prevention of disease progression even in adults.[183] Transplantation of fetal striatal neurons into fetal and neonatal rodent striatum, as well as into adult lesioned striatum, have shown migration, incorporation and target innervation,[184–187] and thus may provide a platform for neuronal replacement and delivery of trophic and anti-apoptotic factors.

Alzheimer's disease (AD)

Transections of the fimbria formix in non-human primates and consequent death of cholinergic neurons in the cortex has been used as a model of AD. Implantation of NGF-producing fibroblasts before lesion protected neurons in the immediate vicinity[188] and did not increase amyloid plaque formation relative to age-matched controls.[189] The global nature of human AD, however, presents problems to neuronal protection on a sufficient scale to envision protection of cognitive functions.

Amyotrophic lateral sclerosis (ALS)

This is a fatal neurodegenerative disease characterized by progressive loss of motoneurons. Based upon studies showing various trophic factors (such as CNTF) can be protective for motoneurons, clinical trials focused on administration of CNTF, but this factor proved toxic when administered systemically. In an effort to deliver trophic factors to ALS patients safely, recent trials have transplanted encapsulated cells genetically engineered to secrete CNTF into the lumbar intrathecal space.[190] Levels of CNTF were detected in CSF for 17 weeks (undetectable before grafting) with no side-effects, but no notable therapeutic effects were observed.

Viral vectors have also been investigated for ALS, and several routes of administration have been analyzed: intramuscular and intravenous injection of Ad encoding CNTF to mutant mice with progressive motor neuropathy (*pmn*) increased their lifespan and reduced neuronal degeneration, whereas intracerebroventricular injection was ineffective.[191] BDNF or GDNF protected motoneurons from axotomy-induced death in neonatal rats.[192] Most recently, myoblasts retrovirally transduced to secrete GDNF were injected into the hindlimb muscles of a 6-week-old transgenic mouse model of familial ALS (SOD1 mutated Gly93Ala).[193] At 18 weeks of age, mice receiving GDNF-producing my blasts showed a higher number of motoneurons when compared with animals receiving control myoblasts. GDNF-producing myoblasts also slowed disease progression and delayed the development of motor abnormalities in these mice.[193]

Ischemia

The degeneration that results from ischemic insult, (compromise of blood flow in the brain or hemorrhagic toxicity) begins with functional impairment and progresses to morphological damage. The lack of oxygen delivery to cells of the brain induces energy depletion from anaerobic glycolysis, release of excitatory amino acid, extreme alterations in Ca+ and Na+ homeostasis, and free radical damage (for review see Pechan *et al*[194]). Several therapeutic modalities for gene therapy have been investigated, including growth factors, anti-apoptotic molecules and neurotransmitter augmentation.

Evidence of apoptosis as a mechanism of cell death in ischemia led investigators toward inhibiting this process to slow or halt the damage from ischemia. Delivery of bcl2 via an HSV amplicon vector, either before or after ischemic insult, protected neurons from cell death induced by ischemic injury,[195,196] and administration of an interleukin-1 receptor antagonist via an Ad vector significantly reduced infarct volume and decreased inflammatory response after focal ischemia.[197,198] Expression of a neuronal apoptosis inhibitory protein (NAIP) via an Ad vector reduced the extent of ischemic damage in rat hippocampus.[199] Expression of 72-kDa heat shock protein via HSV vector also improved neuron survival against transient focal ischemia.[200] Ad vectors have also been shown effectively to transfer genes to cerebral blood vessels and overlying meninges,[201] and transfer of genes for enzymes with vasodilator function may also attenuate ischemic damage. *Ex vivo* therapy for ischemia includes transplantation of fibroblasts genetically modified to secrete NGF into the hippocampus; which protected neurons within CA1 and CA2 regions from damage.[202]

Brain tumor therapy

As with most new therapeutic modalities, cancer is a prime target based on the high incidence and life-threatening aspects of this type of disease. Brain tumors span a range of phenotypes, including astrocytomas, meningiomas and glioblastomas, the latter of which represents 50% of brain tumors in the adult population and currently have a dismal prognosis. Although these tumors remain confined to the brain, they have proven resistant to surgical, drug and radiation therapies due to their invasive nature within the brain, the substantial fraction of tumor cells in temporary growth arrest; the genotypic heterogeneity of tumor cells, the difficulty in delivery of drugs from the blood stream into the brain, and the poor immune surveillance in the CNS. Gene therapy strategies have sought to complement current clinical therapies to increase their potency and extend their range, and include a wide spectrum of therapeutic genes and delivery strategies.

Since the efficiency of gene delivery is the rate limiting

factor *in vivo*, the transgene must confer a 'bystander' effect, such that transduced tumor cells can kill neighboring, nontransduced tumor cells. Modes of delivery should extend the area of gene delivery, at the same time including mechanisms to target tumor cells selectively, and thus spare normal cells. In this latter category, tumor cells within the brain, and the endothelial cells involved in their angiogenesis, represent essentially the only dividing cell population in the adult brain and thus vectors/drugs which are operational only in cycling cells will be intrinsically selective, eg retrovirus vectors and drugs which kill by disruption of DNA replication. There are also other genetic and phenotypic properties of tumor cells which make them distinct targets, including overexpression or mutational activation of growth factor receptors, mutation of growth regulatory proteins (like p53), dependence on neovascularization, and expression of unique mutant proteins that can act as tumor-specific antigens. Further, therapeutic transgene products should act in a combinatorial and/or synergistic manner with each other and with current clinical therapies, for example by injection of vectors in the margins of the tumor resection cavity, use of radiation activated promoters, and enhancement of commonly used chemotherapeutic agents.

As general categories, transgenes have been designed to activate prodrugs, enhance chemotherapeutic agents, block angiogenesis, inhibit tumor cell migration, inhibit cell division, promote immune response to tumor antigens, and directly kill cells. The following are given by way of example out of a rich literature (for review see Kramm *et al*;[203] Weyerbrock and Oldfield[204]). One of the first therapeutic transgenes to be used was the HSV-thymidine kinase gene, as this enzyme can convert ganciclovir to a toxic nucleotide analogue which disrupts DNA synthesis leading to cell death.[205] This prodrug activation mode continues to be a mainstay of brain tumor therapy, but is limited by the antigenic nature of the gene product, which can lead to nonspecific or premature death of cells expressing it, and by the non-diffusibility of the nucleotide analogue, such that it can only be transferred between cells by gap junctions. In the intervening years the number of prodrug activation/drug enhancement strategies has expanded rapidly to include, for example: bacterially derived cytosine deaminase activation of 5-fluorocytosine to 5-fluorouracil, the latter being a commonly used chemotherapeutic agent,[206] which acts synergistically with ganciclovir;[207] mammalian cytochrome P450 2B1 activation of cyclophosphamide, a chemotherapeutic agent normally activated in the liver, but with poor transfer of the active metabolites across the blood–tumor barrier;[208] and the mammalian deoxycytidine kinase gene that activates cytosine arabinoside.[209] Antiangiogenesis inhibitors can be used to block the growth of tumors, with examples including an antagonist for Tie2[210] and a dominant-negative acting VEGF receptor.[211] Tumor cell migration has been restrained by anti-sense blockade of the expression of beta-integrin[212] and fucosyltransferase.[213] Some tumor types respond to growth factors, for example, NT3 can cause terminal differentiation/ apoptosis of medulloblastoma cells.[214] Immune enhancement has been effected by *ex vivo* vaccination paradigms and direct inoculation of cytokine-expressing cells into the brain. In the *ex vivo* methods, tumor cells are removed from the patient, transduced in culture with cytokine genes, and killed by irradiation before peripheral inoculation. A number of cytokines have proven effective in this regard, including GM-CSF.[215–217] Expression of cytokines within the context of the brain tumor, however, can promote an inflammatory response which is toxic to normal cells. Numerous means have also been devised to directly kill tumor cells. This includes use of replication-conditional viruses that kill tumor cells by lytic replication, including E1b-minus adenovirus, which replicates preferentially in cells with mutant p53,[218] and TK-minus and ribonucleotide reductase-minus HSV, which require compensatory cellular enzymes up-regulated in dividing cells to replicate.[5,219] Other weapons include: activation of the tumor necrosis factor receptor[220] or other ligands which trigger apoptosis; antisense against telomerase, which protects the ends of chromosomes;[221] targeting of a glioma-specific chloride channel,[222] and fusion of adjacent cells to form a multinucleated, necrotic mass.[223] Gene therapy offers potential ways to tackle both the invasive nature of glioblastoma and their unique properties, and one can envision a multicomponent therapeutic strategy (Figure 3).

Other disorders

Lysosomal storage disease

Lysosomal storage disorders are inherited diseases marked by deficiencies of lysosomal enzymes. This loss results in the storage of undegraded substrates in the lysosomes in all cells, and consequent dysfunction and death, with therapeutic intervention required in both peripheral tissues and the brain. Importantly, lysosomal enzymes can be released by one cell and taken up by another via an endocytosis process, thus providing a basis for cross-correction strategies. A mouse model of mucopolysaccharidosis type VII (MPS VII) resulting from a mutation in the gene for beta-glucuronidase (GUS) has been the most commonly used model to study different gene therapy approaches to lysosomal disorders affecting the brain.[224] Restoration of GUS activity in the brain was attempted by directly injecting the HSV or Ad vectors encoding GUS into the cornea or striatum, respectively.[225,226] In both treatments the expression of the enzyme was limited to a few specific neuronal areas, although the intrastriatal injection resulted in the widespread presence of enzyme activity within the neocortex. Other experiments have attempted to extend the spatial range of transgene expression within the brain by using an immortalized neural progenitor cell line that can migrate within the brain and differentiate into neurons and glia. These cells were infected in culture with a retroviral vector carrying the GUS gene and grafted into the lateral ventricle of newborn mouse brain.[127] Histochemical analysis revealed the presence of GUS activity and reduction in lysosomal storage material in many brain regions, compared with untreated mutant mice, up to 8 months after cell engraftment. More recently, intrathecal injection of AAV carrying the GUS gene proved effective in increasing GUS activity in brain up to 3 months after injection.[227,228]

Pain

Intrathecal and intraparenchymal delivery of viral vector are a promising modality for treatment of pain. In rat

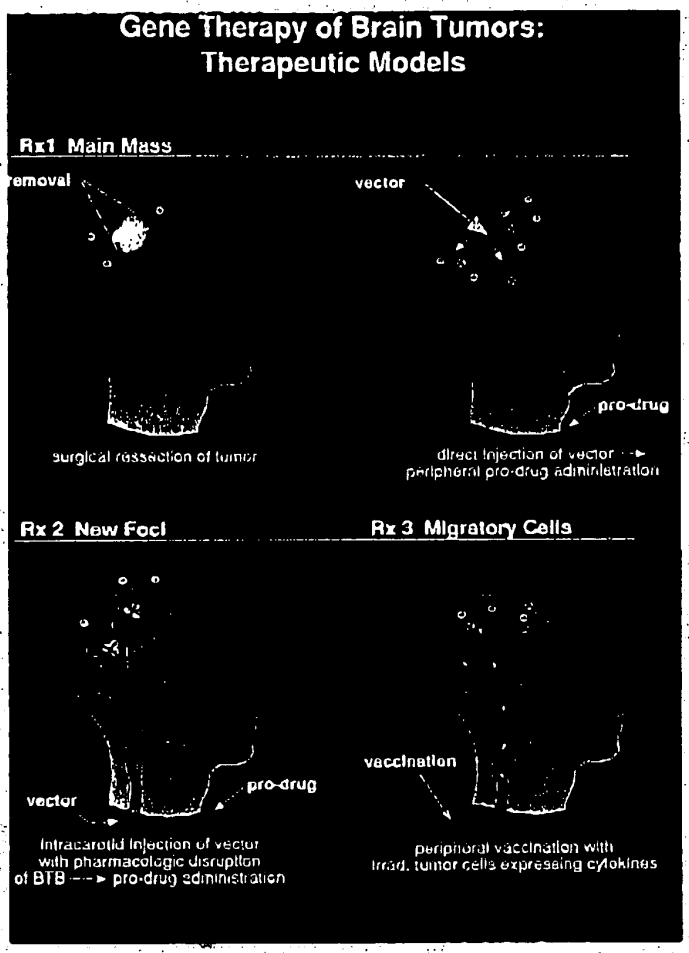

Figure 3 Multicomponent model of brain tumor therapy. The main tumor mass would be removed through neurosurgical procedures with injection of replication-conditional vectors, migratory vector producing cells and/or replication-defective vectors into the resection cavity. Some viable tumor tissue would be placed in culture for ex vivo vaccination at a later time. Injected vectors could carry any of a variety of therapeutic genes; hopefully with additive/synergistic, selective and bystander effects. Subsequently, patients would be treated systematically with appropriate prodrugs/drugs, whose potency would be activated by the transgene products. Assuming that, with time, escapee tumor cells would generate new neovascularizing tumor foci, these would be accessed through temporary disruption of the brain–tumor barrier and vector-mediated transgene delivery, followed by systemic prodrug/drug treatment. With consequent reduction in tumor load, the immune system would then be activated towards tumor antigens using an ex vivo vaccination paradigm.

models, CSF administration of recombinant Ad encoding an endogenous opioid, beta-endorphin, resulted in transduction of pia mater cells, the most internal layer of meninges.[229] The transduced cells released beta-endorphin into the CSF, allowing access of the neuropeptide to opioid receptors, which are widely distributed in the spinal cord and brain (including laminae I and II of spinal cord), and consequently attenuated inflammatory hyperalgesic pain. Delivery of the gene for preproenkephalin into the amygdala using a recombinant HSV vector also resulted in robust, albeit short-term, inhibition of pain.[230,231]

Retinitis pigmentosa (RP)

This is an inherited eye disorder which can be caused by disruptive mutations in the cyclic GMP phosphodiesterase beta subunit (PDE) gene.[232] The lack of expression of this gene in rod photoreceptor cells in the retina results in their degeneration, leading to progressive blindness. The *rd* mouse shows a rapid postnatal degeneration of rods and provides a useful model for studying gene transfer strategies in this disease. Efforts to replace gene function or protect against the degeneration in *rd* mice have focused on three approaches: (1) transfer of the cDNA encoding PDE; (2) transfer of gene encoding CNTF to promote the survival of photoreceptors;[233] and (3) transfer of anti-apoptotic genes to inhibit degeneration of rods.[234] The PDE gene has been delivered to the subretinal space using both AAV and Ad vector.[76,235,236] The use of Ad vectors to deliver PDE gene resulted in a shorter (6 weeks) therapeutic effect[235] compared with the use of gutless Ad vectors (12 weeks).[76] The transient expression

of the PDE gene using Ad vector probably results, in part, from immune response to viral antigens.[237] In recent studies, the delivery of the PDE gene using a lentivirus vector resulted in the sustained expression of the transduced gene for up to 6 months and increased survival of photoreceptors throughout the retina.[238] This prolonged expression of PDE gene was sufficient to maintain one to three rows of rods in the retina of animals injected with vector (out of a normal complement of seven rows), while no rods were present 6 weeks after birth in untreated *rd* animals.

Epilepsy

With the identification of multiple genes causing epilepsy,[239] efforts have begun to focus on gene therapy treatment. Exploratory modalities have included: delivery of heat shock protein (HSP72) via an HSV amplicon vector which protect hippocampal neurons from kainic acid toxicity;[200] Ad-mediated delivery of glutamic acid decarboxylase to increase synthesis of the inhibitory neurotransmitter GABA;[240] and lipofectin-mediated delivery of cholecystokinin, an anticonvulsant and anti-opioid neuropeptide, to the ventricles to alleviate (temporarily) audiogenic seizures.[241]

Endocrine functions

Viral vectors have also been used as tools for examining the role of hormonal replacement therapy in distinct neuroendocrine and/or sensory pathways. Brattleboro rats have no functional vasopressin, an antidiuretic hormone normally synthesized in the hypothalamus and released into the circulating blood in the posterior pituitary, leading to polyuria and polydipsia. Ad vectors encoding vasopressin were delivered into the specific nuclei of the hypothalamus of Brattleboro rats, and functional recovery was observed within a week after gene delivery and persisted for 2 months.[242] Neuropeptide delivery has also been tested in the obese (*ob/ob*) mouse model with AAV delivery of leptin to the brain producing long-term weight loss.

Conclusions

Ongoing discoveries of the cellular and molecular basis of CNS disorders, and continued advancements in gene transfer technology, have contributed to the development of therapies for CNS disorders in animal models that can relieve symptoms, and slow or halt the progression of the disease. Advances in the genetic makeup of vectors and strategic means of delivery have provided selective and global delivery modalities, with some limitations. Since each disease and brain region has special features, the choice of vectors and routes of delivery will need to be tailored accordingly. Continued communication between clinicians and basic researchers is necessary to ensure the proper direction and evaluation of these studies toward therapeutic use. Several studies have been initiated for phase 1 clinical safety trials of gene delivery to the brain, including brain tumors and ALS. However, many improvements in safety, efficacy, stability and regulatability of gene transfer to the brain will need to be made before effective clinical therapy for CNS protection and repair is a reality.

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